Egg Maturation in Laboratory-reared Females of the Swallowtail Butterfly、Papilio xuthus L.(Lepidoptera: Papilionidae)、Feeding on Different Concentration Solutions of Sugar

著者	Watanabe Mamoru
雑誌名	Zoological science
巻	9
号	1
ページ	133-141
発行年	1992-02
権利	(C) 1992 Zoological Society of Japan
URL	http://hdl.handle.net/2241/100340

Egg Maturation in Laboratory-reared Females of the Swallowtail Butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae), Feeding on Different Concentration Solutions of Sugar

MAMORU WATANABE

Department of Bıology, Faculty of Educatıon, Mıe Unıversıty
Tsu-shı, Mıe 514, Japan

Reprınted from
ZOOLOGICAL SCIENCE Vol 9 No 1 (1992)
p 133–141

Publıshed by the
Zoologıcal Socıety of Japan
February 15, 1992

ZOOLOGICAL SCIENCE **9** 133–141 (1992)

Egg Maturation in Laboratory-reared Females of the Swallowtail Butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae), Feeding on Different Concentration Solutions of Sugar

MAMORU WATANABE

Department of Biology, Faculty of Education, Mie University
Tsu-shi, Mie 514, Japan

ABSTRACT—In the laboratory, adult females of the swallowtail butterfly, *Papilio xuthus,* were given sugar solutions of different concentrations. Weight loss of the unfed females was directly proportional to time since emergence. They died within 8 days. Females given 0%, 0.1% or 1% solution of sugar lost weight with ageing. Females feeding on a 10% solution of sugar maintained weight for 15 days after emergence. Conversely, females given 20% or 50% sugar solutions were found to make a gain of 20% or 60%, respectively, in weight during the same period. Newly emerged females took little quantity of 0%, 0.1% or 1% solution of sugar, but their intake increased with ageing. On the other hand, they took over 100 mg of 10%, 20% or 50% solution of sugar. The daily changes to mature eggs in the females were compared among those treated with different foods. Almost no mature eggs were added in the females on feeding less than 1% solutions of sugar. When females took more than 10% solutions of sugar, they produced significantly more mature eggs than those unfed or those who took only water. In older adults, there was a relation between the added number of mature eggs and the accumulated sugar intake. Females may allocate their available energy (fat body+sugars) for egg maturation and body maintenace.

INTRODUCTION

Nectar intake has been shown to increase longevity and egg production in many lepidopteran insects [1–2]. Although the sugar concentration of the nectar in flowers partly depends upon weather conditions, it seems to be maintained within a range between 15% and 45% [3–4]. Three sugars (monosaccharide fructose, glucose and disaccharide sucrose) are generally found in the nectar of many plant species [5–6]. Their sugar concentration and composition vary not only among plant species but also with the age of a single flower [4, 6]. The nectar also includes a little amino acids [7].

Sulfurs, *Colias alexandra* and *C. meadii,* prefer nectar with low sugar concentration, chiefly those including monosaccharides [5]. The energy intake efficiency for some butterfly species is maximised at about 40% sucrose solution [8–10]. In some moths, relationship between the concentration of sugar and fecundity or longevity has been studied [11]. However, there are few reports in butterflies on the feeding regimes related to fecundity and survival depending upon the concentration level of sugar in nectar [12]. The achievement of full reproductive potential during the adult stage may be affected by the level of sugar concentration in the nectar. Therefore, the nectar feeding habit may have come to be evolved in the lifetime reproductive strategy, as well as mating behavior.

The longevity of the swallowtail butterfly, *Papilio xuthus,* has been studied under natural conditions [13], but no information exists on the effects of adult nutrition. Multiple matings in *P. xuthus* increase fecundity [14, 15]. In this paper, I present data from laboratory-reared virgin females of *P. xuthus* subjected to 5 different sugar solution concentrations as a diet and compare their potential fecundity.

MATERIALS AND METHODS

P. xuthus in this study was collected principally

Accepted October 14, 1991
Received June 12, 1991

in Mie Prefecture, in the warm-temperate zone of Japan The females were obtained from a stock culture reared in the laboratory at room temperatures and under a long-day photoperiod (more than 15 hr of light) in the summers of 1986, 1988, 1989 and 1990

As soon as the females emerged, their forewing lengths and fresh weights were measured Then, without mating, each female was assigned to one of seven groups

(1) Females were kept unfed,
(2) Females were fed with distilled water,
(3) Females were fed with a 0 1% solution of sugar,
(4) Females were fed with a 1% solution of sugar,
(5) Females were fed with a 10% solution of sugar,
(6) Females were fed with a 20% solution of sugar,
(7) Females were fed with a 50% solution of sugar

The sugar solution included equal amounts (weight) of fructose, glucose and sucrose Except unfed females, they were supplied with sugar solution or water for only 3 min a day Each female was kept in a glassine envelope and stored in a chamber at ca 25°C The amount of intake was determined as the odds of the weight between pre- and post-feeding, using a semi-microbalance accurate up to 0 1 mg

On death, females from each of the 7 groups had their abdomens immediately fixed in 50% ethyl alcohol, though most of them were able to survive over 15 days after emergence Following dissection, eggs in ovaries were classified into three stages A detailed description of the egg at each stage is given elsewhere [14] The numbers of mature and submature eggs were counted directly The total number of immature eggs was estimated by multiplying the number in one ovariole by eight, that is the number of ovarioles The diameter of mature eggs was also measured under a dissecting microscope

All means are shown with their standard errors

RESULTS

Body size and daily changes in relative weight

The total number of females used for this study was 122 It is commonly observed that the size of adult butterflies, as reflected by the length of the forewing, tends to be smaller in laboratory-reared individuals than that of adults from natural populations Such tendency was also found in *P xuthus* females [15] Accordingly, to minimize the effect of small body size, only females with a forewing length of more than 50 mm were used As shown in Table 1, the mean forewing length in each group was around 56 mm Consequently, they were not significantly different from the forewing length of females collected from natural populations [14]

After emergence, most females evacuated excrement during wing extension Although forewing lengths were mostly stable (Table 1), the fresh weights of 0-day-old females were very variable, possibly because some females held onto excrement until they were weighed

The abdominal cavity of the females just after

TABLE 1 Forewing lengths and fresh weights of 0-day-old females used in the experiment of 7 groups

Group	Forewing length (mm)	Fresh weight (mg)
(1) unfed	55 8±0 4 (n=21)	690 5±26 5 (n=19)
(2) water feeding	56 7±0 9 (n=11)	712 3±30 6 (n=11)
(3) 0 1% sugar solution	55 9±0 8 (n=15)	624 1±14 9 (n=15)
(4) 1% sugar solution	56 3±0 7 (n=16)	616 6±17 0 (n=16)
(5) 10% sugar solution	55 8±0 5 (n=19)	631 7±26 9 (n=20)
(6) 20% sugar solution	56 4±0 5 (n=18)	648 9±18 7 (n=19)
(7) 50% sugar solution	56 4±0 7 (n=15)	740 6±37 2 (n=15)

n number of females examined

emergence was mostly filled with fat bodies and with relatively small air sac Since the fat body was used for the eggs developed, the fat body gradually depleted and the volume of air sac increased with ageing so that it came to fill half the abdominal cavity in 10-day-old females

Unfed females lost weight in proportion to length of time after emergence All died within 8 days (Fig 1) The fresh weight of 8-day-old females just after death was only 43% of that of 0-day-old ones Mean daily weight loss was calculated at about 50 mg

The weight of each female supplied with water or sugar solution was based on the weight of pre-feeding Females fed on distilled water also became lighter as they age (Fig 1) There are no difference in relative weight between unfed and the water feeding females in the first 3 days

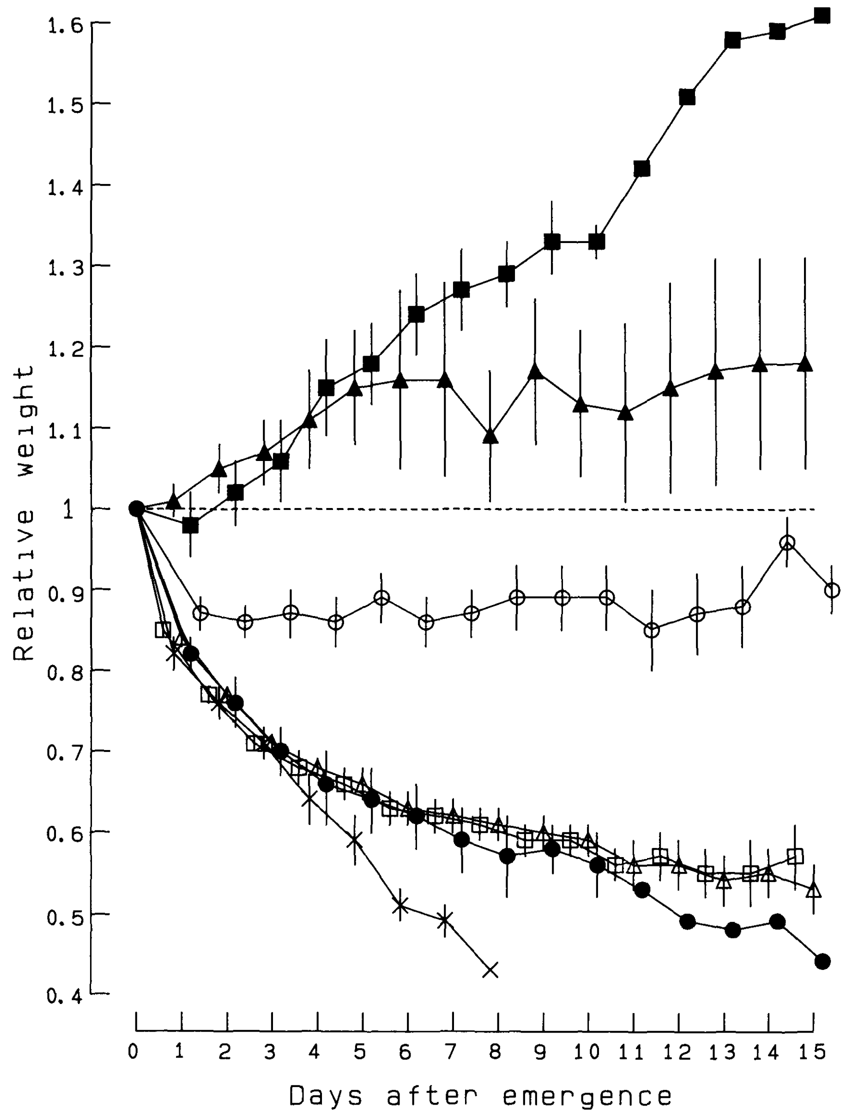

FIG 1 Change in the relative weight of unfed females (cross) and females feeding on water (solid circle), on sugar solutions of 0 1% (open square), 1% (open triangle), 10% (open circle), 20% (solid triangle) and 50% (solid square)

However, the weight loss was lower than that in unfed females thereafter Some of them survived 15 days after emergence, when the mean fresh weight was about 44% of that in 0-day-old females Mean daily weight loss was calculated at about 27 mg Therefore, 23 mg of distilled water was daily retained in the female body

Females fed on 0 1% or 1% solution of sugar became lighter as they age Although the decline in their weight was similar to that in females fed distilled water up to 10-day-old, all were able to survive 15 days after emergence Their weight was more than half of that in females just after emergence Therefore, it can be said that such sugars, whilst not maintaining the female's weight, contributed to their longevities

Females fed on 10% solution of sugar kept their weight constant throughout the experimental period Naturally, all were able to survive after the end of the experiment, 15 days after emergence

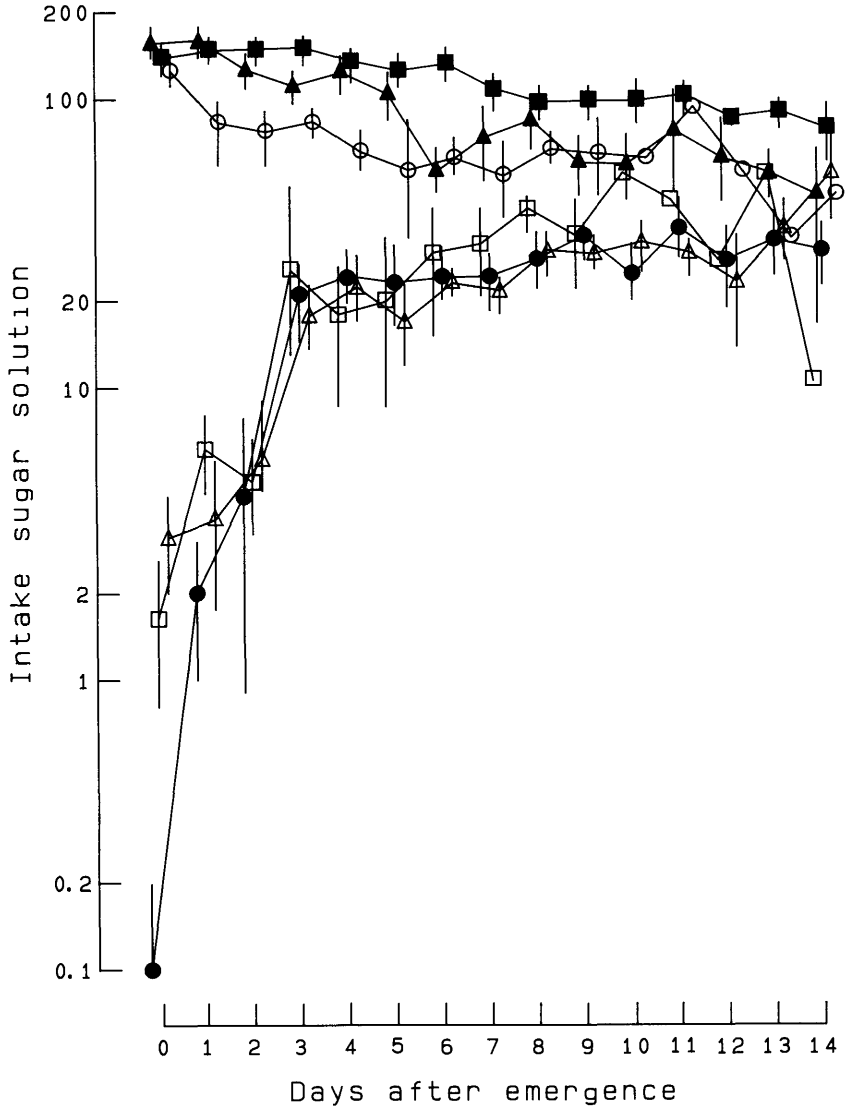

FIG 2 Change in the intake of water (solid circle), sugar solutions of 0 1% (open square) and 1% (open triangle), 10% (open circle), 20% (solid triangle) and 50% (solid square) [mg, ±SE]

Such sugars are effective as an energy resource for maintaining body weight

Females fed on 20% or 50% solution of sugar became heavier as they age Their respective weights after 15 days was 1 2 and 1 6 times heavier than those of females just after emergence Consequently, high sugar concentration enhances longevity and body weight in female adults, though crystals of sugar were found in the hindgut, sometimes in the midgut of the females feeding on 50% solution of sugar

Amount of daily sugar intake

Newly emerged females (=0-day-old) took little water (Fig 2) Mean water intake by females was about 0 1 mg Besides water, they also took a little quantity of 0 1% (ca 1 5 mg) or 1% (ca 4 mg) solution of sugar The mean sugar intake by females in the 0 1% or 1% group was 0 001 mg and 0 04 mg, respectively The water intake by 1-day-old females increased to 2 mg Females in the 0 1% and 1% group also took more sugar solution than 0-day-old ones The water intake by females increased as they age and ultimately reaching about 40 mg Although intake of 0 1% or 1% solution of sugar by females was somewhat higher than that of water in younger stages, daily changes in intake of such sugar solutions were similar to those in intake of water This means that females only took 0 04 mg and 0 4 mg of sugar daily from 0 1% and 1% solution, respectively Therefore, at least the feeding response by older females to sugar solutions did not differ from that to water Females may be unable to distinguish the sugar solution of less than 1% from water

Females of 0-day-old took about 140 mg of 10% solution of sugar, or 14 mg of sugar (Fig 2) Although the 20% sugar solution was taken in large quantity (ca 160 mg), the intake by 0-day-old females was almost the same for 10%, 20% and 50% sugar solutions Also, daily intake by females of more than 10% sugar solutions gradually decreased as they age 14-day-old females took less than 100 mg The respective sugar intakes were calculated as ca 8 mg, 10 mg and 25 mg for the females took 10%, 20% and 50% solutions

Daily changes in the number of eggs

The diameter of mature eggs in the oviducts was about 1 27 mm (min 1 183 mm and max 1 400 mm) It remained constant irrespective of female age and diet

In the present study, the number of eggs in 0-day-old females was 0 3±0 3 (n=4), 52 5±1 7 (n=4) and 706 3±34 1 (n=4) for mature, submature and immature eggs, respectively Since the unfed females or those supplied with only water were never nourished, the number of eggs in the two groups were averaged together Table 2 shows that the number of mature eggs increased toward 20 in 3-day-old females The number of submature eggs decreased at the 3rd day after emergence, and then the number did not increase Therefore, few submature eggs seem to be added from immature eggs in the females The number of immature eggs did not change throughout the life span of adults

The daily changes in the number of eggs were compared among the females of different feeding treatments If sugars contribute to egg maturation, the number of mature and submature eggs might be expected to increase

Although only small sample sizes were available during younger stages, almost no mature eggs were added to the females treated with 0 1% and 1% sugar solutions (Table 2) However, in females fed 10% sugar solution, the number of mature eggs was significantly higher than that of unfed females and those fed only water Such a phenomenon was also observed among females treated with 20% and 50% sugar solutions After 15 days, some retained more than 150 mature eggs There seemed to be no changes in the number of submature and immature eggs irrespective of sugar concentrations (Table 2) Since many mature eggs were added from submature eggs, the females must make up for 'the loss' of submature eggs

Differences in sugar concentrations affects daily sugar intake in each butterfly If the amount of sugar was effective for females to produce excess eggs comparing to females given no sugar, the number of mature eggs would be related to the amount of accumulated sugar intake During younger stages, there seemed to be no relationship between the number of mature eggs and the

TABLE 2 Change in the number of eggs (three stages) in females feeding 0 1%,

	Days after emergence	Unfed and water feeding		0 1% sugar solution	
No mature eggs	0	0 3± 0 3	(4)	—	
	1	6 6± 3 0	(5)	17	(1)
	2	15 0± 4 9	(3)	40	(1)
	3	18 2± 6 0	(5)	4	(1)
	5	14 6± 2 3	(5)	5	(1)
	6~15	18 8± 7 9	(4)	3 4± 2 2*	(7)
No submature eggs	0	52 5± 1 7	(4)	—	
	1	40 8± 9 6	(5)	42	(1)
	2	82 3± 21 1	(3)	68	(1)
	3	36 0± 6 6	(5)	36	(1)
	5	36 2± 2 4	(5)	35	(1)
	6~15	34 3± 12 8	(4)	11 3± 5 3	(7)
No immature eggs	0	706 3± 34 1	(4)	—	
	1	872 4± 65 9	(5)	912	(1)
	2	963 0± 81 8	(3)	806	(1)
	3	1029 4± 86 1	(5)	699	(1)
	5	837 0± 31 8	(5)	814	(1)
	6~15	737 3±129 1	(4)	494 7± 85 1	(1)

( ) number of females examined
* 0 05>P>0 01
** P<0 01

TABLE 3 Regression coefficient (b) and determination coefficient (r^2) in the log number of mature eggs on the log amount of accumulated sugar intake

Days after emergence	b	r^2	n	t	
1	0 140	0 25	11	1 734	n s
2	0 123	0 20	10	1 407	n s
3	0 373	0 72	10	4 530	P<0 01
5	0 416	0 96	12	15 692	P<0 01
10	0 358	0 32	10	1 925	0 1>P>0 05
15	0 749	0 77	12	5 866	P<0 01

n number of females examined

accumulated sugar intake (Table 3) Egg maturation in younger females probably depended on the nutrition reserves carried over from the larva rather than foods taken after emergence The fat body seems to be a major energy resource for the maturation of eggs and for maintenance of adult life However, since the fat body decreased as they age, older females would depend more heavily on the sugar as an energy resource Accordingly, it seems to be reasonable that the regression coefficients (b) and the determination coefficients (r^2) in the relation between the number of mature eggs and the amount of accumulated sugar intake by females increased

DISCUSSION

In nature, the mean longevity of the adult swallowtail butterflies was estimated at 16 2 days for *P xuthus* [13], 12 7 days for *P polytes* [16] and

1%, 10%, 20% and 50% comparing with those in unfed and water feeding females

1% sugar solution		10% sugar solution		20% sugar solution		50% sugar solution	
—		—		—		—	
0	(1)	18 7± 10 0	(3)	42 0± 3 6**	(3)	34 7± 14 8	(3)
13	(1)	38 0± 17 9	(3)	87 5± 1 5**	(2)	67 7± 20 2	(3)
1	(1)	44 3± 19 9	(3)	89 3± 12 4**	(3)	99 5± 9 5**	(2)
7 5± 0 5	(2)	80 3± 3 0**	(3)	122 7± 12 2**	(3)	92 7± 14 8**	(3)
0 8± 0 6**	(8)	126 3± 13 3**	(4)	127 8± 42 1*	(4)	159 8± 19 5**	(4)
—		—		—		—	
25	(1)	78 0± 24 6	(3)	44 3± 21 9	(3)	80 7± 23 1	(3)
12	(1)	61 0± 10 7	(3)	115 5± 21 5	(2)	73 7± 8 3	(3)
37	(1)	46 3± 10 5	(3)	68 7± 16 5	(3)	50 0± 14 0	(2)
20 5± 6 5*	(2)	74 7± 7 9**	(3)	50 3± 10 3	(3)	65 0± 20 7	(3)
15 6± 6 1	(8)	81 8± 14 1*	(4)	90 3± 55 7	(3)	84 0± 6 9*	(4)
—		—		—		—	
793	(1)	735 7± 87 0	(3)	789 0± 73 0	(3)	762 7± 91 0	(3)
1160	(1)	669 3± 32 7*	(3)	777 5± 68 5	(2)	834 7± 54 3	(3)
804	(1)	839 0± 62 7	(3)	828 3± 11 4	(3)	830 5± 11 5	(2)
761 0±232 0	(2)	828 3± 30 1	(3)	883 7± 88 3	(3)	883 7± 56 0	(3)
553 5± 40 2	(8)	996 8±167 3	(4)	735 0±111 7	(3)	764 8±123 0	(4)

16–17 days for *P helenus* and *P protenor* [17] All of them relied on nectar of many flowering plants for their energy resource [18]

In this experiment, as I kept the females in the envelope all day except feeding time, so that each adult consumed the least amount of energy to maintain life Unfed females survived for 8 days after emergence, after which no fat body was found in their body This shows that the energy derived from the larval stage can support about 1 week of life in a motionless adult

Water intake enhanced the longevity of the checkerspot butterfly, *Euphydryas editha* [2] As for *P xuthus,* some females taking water survived for 15 days, and their daily weight loss was lower than that of unfed females after 4-day-old Therefore, the water derived from the larval stage was likely to be effective during the first 3 days The daily difference was about 28 mg, most of which would be attributable to water loss from the body

There were no significant differences in changes of body weight among females taking water and less than 1% solutions of sugar Then, all the females in the groups survived for 15 days, suggesting that the sugar enhanced longevity In order to release the cue for adult feeding, Murphy *et al* [2] added a trace of sugar into the water as a diet In the present study, however, the degree of the cue for feeding less than 1% sugar concentration was similar to that for taking water Therefore, the trace sugar seemed to be important in maintaining the female body, though fat body was mostly exhausted at 15-day-old

Females feeding on a 10% sugar solution maintained their body weight until 15-day-old The amount of sugar intake seems to accord with the loss for respiratory metabolism and maintenance of body On the other hand, females feeding a 20% and 50% sugar solutions increased their body weight The fat body was found in the females of 15-day-old, suggesting that sugar played a role in substituting for the fat body The fact that crystals of sugar were found in the gut of the females feeding on 50% solution of sugar suggested, however, that the 50% sugar solution was not a suitable concentration

Fat body is nutrition reservoir derived from the larval stage and is a resource for egg maturation during the adult stage [19] This partly means that the number of mature eggs produced was determined by the amount of fat body at emergence In the present study, even unfed females were able to produce some mature eggs, probably using the energy derived from the fat body Since the number of mature eggs were almost the same among 1-day-old females except those feeding on a 20% sugar solution, the energy resource for egg maturation in younger females was not sugar but either the fat body or other substances, both of which were derived from the larval stage

There was a rapid maturation of eggs in more than 2-day-old females feeding more than 10% sugar solutions, though some data were not significantly different Since unfed and water feeding females maintained a certain number of mature eggs with depletion of fat body, females must allocate their available energy (fat body+sugars) for egg maturation and body maintenance Further oogenesis was found in females feeding on higher concentrations of sugar solutions

The available energy did not affect the size of mature egg in *P xuthus* In nature, the egg size did not change as they age [14] Murphy *et al* [2] pointed out that amino acid intake leads to heavier eggs, larvae from which are more likely to survive However, Karlsson and Wiklund [20] showed that the egg size did not affect the survival rate of larvae

Watanabe [15] showed that multiple matings in *P xuthus* increased the number of eggs deposited This phenomenon suggested that a certain substance derived from mating was used for egg maturation On the other hand, flower nectar contains amino acids [4] and has been shown to be an important food resouce in lepidopteran insects [2, 7] Therefore, the relative importance of these resources varies with the mating performance, though the primary adult food source is flower nectar [21]

The most effective sugar concentration was 40% in *Thymelicus lineola* [9] In the present experiment, a 50% sugar solution was the most effective concentration for egg maturation However, some sugars were not absorbed and crystallized in the guts, probably affecting the digestive organ It would be of interest to attempt to correlate the sugar concentration in nectar with the puddling behavior in swallowtail butterflies, though sodium was a stimulus for puddling behavior by *P glaucus* [22]

ACKNOWLEDGMENTS

I thank Dr A Kokubo for critical review of the manuscript, and anonymous referees for giving me useful comments I am also grateful to Miss Y Ueda and Mr F Todo for their assistance This work was supported in part by Grant-in-Aid from the Ministry of Education, Science and Culture, Japan (No 01540545)

REFERENCES

1 Stern, V M and Smith, R F (1960) Factors affecting egg production and oviposition in populations of *Colias philodice eurytheme* Boisduval (Lepidoptera Pieridae) Hilgardia, **29** 411–454

2 Murphy, D D , Launer, A E and Ehrlich, P R (1983) The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha* Oecologia, **56** 257–263

3 Roubik, D W and Buchmann, S L (1984) Nectar selection by *Melipona* and *Apis mellifera* (Hymenoptera Apidae) and the ecology of nectar intake by bee colonies in a tropical forest Oecologia, **61** 1–10

4 Boggs, C L (1987) Ecology of nectar and pollen feeding in Lepidoptera In "Nutritional Ecology of Insects, Mites, and Spiders" Ed by F Slansky, Jr & J G Rodriguez, John Wiley & Sons, pp 369–391

5 Watt, W B , Hoch, P C and Mills, S G (1974) Nectar resource use by *Colias* butterflies Chemical and visual aspects Oecologia, **14** 353–374

6 Watanabe, M , Koizumi, H , Suzuki, N and Kiritani, K (1988) Studies on ecology and behavior of Japanese black swallowtail butterflies VII Nectar of a glory tree, *Clerodendron trichotomum,* as a food resource of adults in summer Ecol Res , **3** 175–180

7 Baker, H G and Baker, I (1973) Amino-acids in nectar and their evolutionary significance Nature, **241** 543–545

8 May, P G (1985) Nectar uptake rates and optimal nectar concentrations of two butterfly species Oecologia, **66** 381–386

9 Pivnick, K A and McNeil, J N (1985) Effects of nectar concentration on butterfly feeding measured feeding rates for *Thymelicus lineola* (Lepidoptera Hesperiidae) and a general feeding model for adult Lepidoptera Oecologia, **66** 226–237

10 Boggs, C L (1988) Rates of nectar feeding in

butterflies effects of sex, size, age and nectar concentration Functional Ecology, **2** 289–295

11 Wigglesworth, V B (1972) The principles of insect physiology 7th ed Chapman and Hall, London

12 Norris, M J (1936) The feeding-habits of the adult Lepidoptera Heteroneura Trans R Ent Soc Lond , **85** 61–90

13 Tsubaki, Y (1973) The natural mortality and its factors of the immature stages in the population of the swallow-tail butterfly *Papilio xuthus* Linne Jap J Ecol , **23** 210–217

14 Watanabe, M and Nozato, K (1986) Fecundity of the yellow swallowtail butterflies, *Papilio xuthus* and *P machaon hippocrates,* in a wild environment Zoological Science, **3** 509–516

15 Watanabe, M (1988) Multiple matings increase the fecundity of the yellow swallowtail butterfly, *Papilio xuthus* L , in summer generations J Insect Behavior, **1** 17–29

16 Watanabe, M (1979) Population sizes and resident ratios of the swallowtail butterfly, *Papilio polytes* L , at a secondary bush community in Dharan, Nepal Kontyu, **47** 291–297

17 Ban, Y , Kiritani, K , Miyai, S and Nozato, K (1990) Studies on ecology and behavior of Japanese black swallowtail butterflies VIII Survivorship curves of adult male populations in *Papilio helenus nicconicolens* Butler and *P protenor demetrius* Cramer (Lepidoptera Papilionidae) Appl Ent Zool , **25** 409–414

18 Suzuki, N , Yamashita, K , Niizuma, A and Kiritani, K (1987) Studies on ecology and behavior of Japanese black swallowtail butterflies 6 Nectar feeding of *Papilio helenus nicconicolens* Butler and *P protenor demetrius* Cramer as main pollinators of glory bower, *Clerodendron trichotomum* Thunb Ecol Res , **2** 41–52

19 Dunlap-Pianka, H , Boggs, C L and Gilbert, L E (1977) Ovarian dynamics in Heliconiine butterflies Programmed senescence versus eternal youth Science, **197** 487–490

20 Karlsson, B and Wiklund, C (1984) Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly *Lasiommata megera* Oikos, **43** 376–385

21 Gilbert, L E and Singer, M C (1975) Butterfly ecology Ann Rev Ecol Syst , **6** 365–397

22 Arms, K , Feeny, P and Lederhouse, R C (1974) Sodium Stimulus for puddling behavior by tiger swallowtail butterflies, *Papilio glaucus* Science, **185** 372–374